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Research Article

PHYTOCHEMICAL ANALYSIS AND SCREENING OF TOTALFLAVONOID, TANNIN AND PHENOLIC CONTENTS INGRACILARIA EDULIS AND HYPNEA VALENTIAE

Divya, S. R and Chandramohan, A*

Department of Biochemistry and Chemistry, Jaya College of Arts & Science, Tamilnadu, INDIA

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A B S T R A C T

The aim is to study the phytochemical constituents present in the two species of algae, Gracilariaedulisand Hypneavalentiae. Preliminary phytochemical analysis revealed the presences of phytochemicals such as carbohydrates, quinines and glycosides in all the tested extracts in both the Gracilariaedulisand Hypneavalentiae. Alkaloids, coumarins, proteins, steroids, phytosteroids, phlobatannins and anthraquinones were absent in all the tested extracts. The amount of total phenolics, total flavonoids and tannin content in ethyl acetate and ethanol extracts of G. edulisand H. valentiaewere determined spectrometrically. The ethyl acetate extract of G. edulisand ethanol extract of H. valentiaeshowed higher flavonoid content. The ethyl acetate extract of G. edulisand H. valentiaeshowed higher total tannin content. The present study suggested that, detailed studies on the isolation and characterization of the seaweed extracts as well as investigations on other biological studies and in vivo assays will be interesting in discovering new drugs.

INTRODUCTION

*Gracilaria*species are a major source of agar, particularly the agar used by the food industry (Santelices and Doty 1989) and approximately 60% of all agar is produced from this alga (Tseng 2001). Gracilarioids are farmed on a large scale in several countries (Skriptsova *et al*, 2009). The technology of *Gracilaria*outdoor cultivation includes usually a nitrogen and phosphate pulse feeding with optimization of the frequency and concentration for high yields (Capo *et al*, 1999). The export price of *Gracilaria*and its derivatives fluctuated around US$ 40 millions in 2002 (Sernapesca 2002). Six species of Gracilaria (*G. edulis, G. crassa, G. foliifera, G. corticata, G. millardetii,* and *G. fergusonii*) occurring in Indian waters have been reported to be potential sources of agar (Kappanna and Rao 1963). *Gracialriaedulis*, a common species of *Gracilaria*has been recommended as source of agar having gel textural attributes suitable for food applications, although inferior in gel quality compared to those harvested species (Villanueva and Montana, 1999).

*Hypneavalentiae*is also a very good source of agar and it is a very popular in alga in developing countries for its secondary metabolites content and it has been investigated as a source of medicinal agents (Murano*et al*., 1998). Elsie *et al,* (2011) reported that ethanol extract of *Hypneavalentiae*showed maximum activity against *Streptococcus aereus*, minimum activity against *Klebsiellapneumoniae*and moderate activity against *Micrococcus luteus*and *Bacillus cereus.* Several works have been undertaken on crude extracts and purified compounds obtained from seaweeds for evaluating their bioactive potential and also screening biologically active compounds of seaweeds against various human pathogenic viruses, bacteria and fungi (Blunded*et al*, 1991; Nascimento *et al*, 1993; Kuda*et al*, 2006; Fusco *et al*, 2007; Chanini *et al*, 2008; Devi *et al*, 2008; Kuda and Ikemori, 2009).

Seaweeds are widely used for therapeutic applications like treatment of tuberculosis, arthritis, colds, influenza, and worm infestations (Smith, 1944). Seaweeds have been adopted into formulas for treating other soft swellings, including ovarian cysts, breast lumps, lymph node swellings, lipomas, and fat accumulation from simple obesity due to its high fiber content (Bensky and Barolet, 1990). Seaweeds have adapted to grow in marine environments from coast to coast, living in both tropical and temperate regions on almost every continent in the world. The rapidly expanding scientific knowledge on seaweeds has led to a growing awareness that seaweeds are valuable coastal resources.

Phenolic compounds are commonly found in the edible brown and green seaweeds in which the antioxidative property has been correlated to their phenolic content (Jimenez-Escrig*et al*, 2001). Although several seaweeds possess wide application in food and pharmaceutical industry, the phytochemical constituents of many types of seaweed in Indian coastal area are still unexplored. The main objective of the present study is to evaluate the preliminary phytochemical constituents, total phenolic content, flavonoid content and total tannin contents of *Gracilariaedulis*and *Hypneavalentiae*.

MATERIALS AND METHODS

Sample collection and preparation of extracts

Healthy, disease seaweeds of *Hypneavalentiae* (0001) and *Gracilariaedulis* (0002) were collected from costal region of Tamil Nadu. Freshly collected sample were transferred to the laboratory in sterile bags.The collected seaweeds was authenticated by Dr. Krishnamurty in (KIA) Krishnamurty Institute of Algology (KIA/2012/DZAB-0001) and (KIA/2012/DZAB-0002). 500 g of powdered algal material (Figure 1 and Figure 2) was extracted by using soxhelet method with different solventssequentially with hexane, petroleum ether, ethyl acetate and ethanol. The extracts were filtered using Whatman filter paper No. 1 and concentrated with rotary vaporator. Which was used for further phytochemical analysis.

Figure 1 Ground sample of *Hypneavalentiae*

Figure 2 Ground sampleof *Gracilariaedulis*

Phytochemical screening

The phytochemical analysis of *Gracilariaedulis* and *Hypneavalentiae*were done to detect the presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, terpenoids, triterpenoids, phenols, coumarins, steroids and phytosteroids, phlobatannins and anthraquinones indicated results as given in the (Table 1 and Table 2) respectively.

Table 1 Phytochemical screening of *Hypneavalentiae*

Tests	Hexane	Petroleum ether	Ethyl acetate	Ethanol
Carbohydrates	+	+	+	+
Tannins	-	-	+	+
Saponin	-	–	+	+
Flavonoid	-	-	+	+
Alkaloid	+	–	–	–
Quinones	+	+	+	+
Glycosides	+	+	+	+
Cardiac glycosides	–	+	+	–
Terpenoids	–	-	+	-
Triterpenoids	–	–	–	–
Phenols	-	-	+	+
Coumarins	–	–	–	–
Proteins	–	–	–	–
Steroids and Phytosteroids	–	–	–	–
Phlobatannins	–	–	–	–
Anthraquinones	–	–	–	–

'+' indicates presence and '-' indicates absence

Estimation of Total phenolic content

Total phenolic content in the ethyl acetate and ethanol extracts of seaweed was determined using the Folin-Ciocalteu reagent method (Miliauskasa *et al,* 2004). This method depends on the reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm using spectrophotometer. Stock solution of algal extracts was prepared to the concentration of 1mg/ml. To 0.1ml of each extract, 5ml of Folin-Ciocalteu reagent was added.

Table 2 Phytochemical screening of *Gracilariaedulis*

Tests	Hexane	Petroleum ether	Ethyl acetate	Ethanol
Carbohydrates	+	+	+	+
Tannins	-	–	+	+
Saponin	-	–	+	+
Flavonoid	-	–	+	+
Alkaloid	+	–	–	–
Quinones	+	+	+	+
Glycosides	+	+	+	+
Cardiac glycosides	–	+	+	–
Terpenoids	–	+	+	+
Triterpenoids	–	–	–	–
Phenols	-	–	+	+
Coumarins	–	–	–	–
Proteins	–	–	–	–
Steroids and Phytosteroids	–	–	–	–
Phlobatannins	–	–	–	–
Anthraquinones	–	–	–	–

'+' indicates presence and '-' indicates absence

The mixture solution was vortexed and incubated in dark for 3 mins. To the incubated content, 5 ml of sodium carbonate (0.75%) solution was added to the above content and mixed thoroughly. The reaction content was incubated in dark for 1 hr. The absorbance was read at 765 nm. Blank was maintained with 5 ml Folin-Ciocalteu reagent, 1 ml ethanol and 4 ml sodium carbonate solution. The concentration of total phenolic content in the extract was expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g) of extract. Gallic acid stock solution was prepared to the concentration of 1 mg/ml. Serial dilution was carried out and gallic acid solution was dissolved in ethanol. A linear dose response regression curve was generated using absorbance reading of gallic acid at the wavelength of 765 nm.

Estimation of Total flavonoid content

Total flavonoid content in the ethyl acetate and ethanol extracts of seaweed was determined using the method described by (Sakanaka*et al,* 2005). The flavonoid content was determined by aluminium chloride method using quercitin as standard. Extracts and quercitin were prepared in ethanol (1 mg/ml). 0.1ml of extract was mixed with 0.9ml of distilled water in test tubes, followed by addition of 75 µL of 5% sodium nitrite solution. After six minutes, 150µL of 10% aluminium chloride solution was added and the mixture was allowed to stand for five minutes after which 0.5 ml of 1M NaOH was added to the reaction mixture. The reaction mixture was brought to 2.5 ml with distilled water and mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. Determination was performed in three replicates. A calibration curve was generated using various concentrations of quercitin (20 - 140µg). Blank consist of all the reagents, except for the extract or quercitin standard solution is substituted with 0.1 ml of ethanol. Results were expressed as mg of quercitin equivalent/g of dry weight (mg QE/g) of extracts.

Estimation of total tannins

Total Tannin content in the ethyl acetate and ethanol extracts of seaweed was determined by Folin–Denis method (Schanderi, 1970) with minor modifications. Stock solution of

seaweed extracts was prepared to the concentration of 1mg/ml. To 0.1ml of each extract, 1ml of distilled water was added and then mixed with0.5 ml of Folin–Denis reagent. The reaction mixture was alkalinized by the addition of 1 ml of 15% (w/v) sodium carbonate solution and kept in dark for 30 mins at room temperature. The absorbance of the solution was read at 700 nm using spectrophotometer and the concentration of tannin in the extract was determined using pure tannic acid as standard (1mg/ml). A calibration curve was generated using various concentrations of tannic acid (20-120µg) was obtained. Blank consist of all the reagents, except for the extract or standard solution is substituted with 0.1 ml of water. Results were expressed as mg of Tannic acid equivalent/g of dry weight (mg TE/g) of extracts.

RESULTS AND DISCUSSION

The results of phytochemical constituents present in the hexane, petroleum ether, ethyl acetate and ethanol extracts of *G. edulis*and *H. valentiae*are presented in table 1.

Phytochemicals such as carbohydrates, quinines and glycosides were present in all the tested extracts of *G. edulis*and *H. valentiae*. Alkaloids, coumarins, proteins, steroids, phytosteroids, phlobatannins and anthraquinones are absent in all the tested extracts. Alkaloids are present in only hexane extract of *G. edulis*and *H. valentiae*. Tannins, saponins, flavonoids and phenols are present in the ethyl acetate and ethanol extracts of *G. edulis*and *H. valentiae*. These compounds present in a variety of medicinal plants have significant application against human.

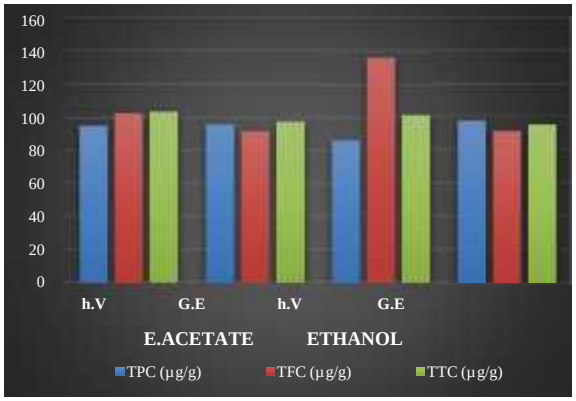

Figure 3 Total Phenol, flavonoids and tannin content in ethyl acetate and ethanol extracts of *Gracilariaedulis*and *Hypneavalentiae*

Pathogens, including those that cause enteric infections and are reported to have curative properties against several pathogens and therefore could suggest their use in the treatment of various diseases (Hassan *et al*., 2004).

Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity in most of the medicinal plants (Mantle *et al*, 2000). Glycosides serve as defence mechanisms against predation by many microorganisms, insects and herbivores (Dhar *et al*, 1979). Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids are reported to have anti-inflammatory effects (Orhan *et al*, 2007; Akhindele *et al*, 2007). Tannins play a major role as antihaemorrhagic agent and showed to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties (Price *et al*, 1987). Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities (Cherian and Augusti, 1995). Steroids, saponins and triterpenoids showed the analgesic properties (Sayyah *et al*, 2004; Malairajan *et al*, 2006).

The amount of total phenolics in ethyl acetate and ethanol extracts of *G. edulis* and *H. valentiae* were determined spectrometrically according to the Folin–Ciocalteu procedure and calculated as gallic acid equivalent (Fig. 3). The amounts of total phenolics content in ethyl acetate *H. valentiae and G. edulis*showed (95.4µg/g and 96.2 µg/g). The ethanol extract of *G. edulis* (97.3µg/g) showed higher phenolic content then *H. valentiae* (86.3 µg/g). The ethyl acetate extract (91.80 µg/g) of *G. edulis*showed higher flavonoid content than ethanol extract (91.10 µg/g); ethanol extract (136.50 µg/g) of *H. valentiae*showed higher flavonoid content than ethyl acetate extract (103.0 µg/g). Ethyl acetate extract of *G. edulis* (104.0 µg/g) and *H. valentiae* (97.60 µg/g) showed higher total tannin content. As phytochemicals often play an important role in plant defense against prey, microorganism, stress as well as interspecies protections, these plant components have been used as drugs for millennia and hence, screening of phytochemicals serves as the initial step in predicting the types of potential active compounds from plants (Chew *et al*, 2011).

Flavonoid compounds especially quercetin and genistein have antitumor activity and these compounds are cytotoxic to cancer cells but have no or insignificant activity in normal cells (Pouget*et al*, 2001). It has been reported that flavonoid, apigenin holds great promise as a chemopreventive agent for a variety of cancers and exhibits significant activity against UV induced DNA damage and thus protect against skin cancer (Baliga and Katiyar, 2006). Plant phenolics are a major group of compounds that act as primary antioxidants of free radical scavengers (Polterait, 1997).

CONCLUSION

The present study of the phytochemical screening, total phenolics, total flavonoids and total tannins of different extracts of *Gracilariaedulis*and *Hypneavalentiae*showed that, these algae could be a potential source for natural antioxidants. It has been reported that most active principles in seaweeds are frequently alkaloids, flavonoids and phenols and these may be responsible for many of the pharmacological actions of the particular plant. If these seaweeds are examined for further biological studies, it could be a promising agent in scavenging free radicals and treating diseases related to free radical reactions. Furthermore, detailed studies on the isolation and characterization of the seaweed extract as well as studies with other models such as lipid peroxidation and *in vivo* assays will be interesting in discovering new drugs.

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